

EFFECTS OF SEMI-STARVATION ON GROWTH AND MORPHOGENESIS DURING THE LARVAL STAGES OF A COMMON MILLIPED, *NARCEUS ANNULARIS* (RAF.)¹

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The life-cycle of the Diplopoda is divided into three phases: the egg, the larva, and the adult. In *Narceus annularis* (Raf.), the common northern spirobolid milliped, the egg is enclosed in a capsule fashioned out of regurgitated leaf litter by the female at the time of egg-laying (Barber, 1915). The first two instars and at least three days of the third instar are spent within the micro-environment of the egg capsule (Berns, 1968). Following emergence from the egg-capsule, the milliped goes through at least seven additional instars before sexual maturity is attained.

In most arthropods, the passage through the larval instars is mainly related to growth in size (Wigglesworth, 1965). The morphogenetic and differentiative processes (with the exception of reproductive structures and regeneration) are mainly confined to the egg, and to the pupal stage in the holometabolous insects. In millipeds, however, passage through the larval instars is not only characterized by growth, but also by a progressive increase in the number of body segments (in *Narceus annularis*, from 7 segments in instar 2, to 51–59 segments in the adult), limbs, and ocelli. Therefore, each instar is a morphogenetic and differentiative phase.

Preliminary observations (Keeton, 1960) suggested to us that environmental factors, particularly food supply, can strongly influence, in a quantitative fashion, the developmental changes occurring in each larval instar. Consequently, we set out to determine the effect of diet on growth, segment addition, and ocelli addition at each instar, as well as the number and duration of the instars. We were particularly interested in discovering which effects of semi-starvation would carry over into the sexually mature adults, and which effects would disappear as a result of developmental compensation. We also hoped to find characteristics that would be reliable in determining the instar of larvae.

MATERIALS AND METHODS

Adult females of *Narceus annularis* were collected from the field and placed in plastic boxes with a maximal supply of leaf litter for food. Nine hundred third instar larvae from eggs laid by these females were used in this study. Four hundred and fifty of these larvae were placed in 15 covered plastic boxes (12" × 4" × 6"), 30 animals per box, with a maximal supply of leaf litter for food. The other 450 larvae were placed in 15 boxes and were given only fecal pellets

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mixed with soil (1:1) for the first six months. After six months, these larvae were given once a month an amount of leaf litter consumable in one week; they were fed only fecal pellets the remaining three weeks of each month. This modification of the original diet was necessary to prevent excessive mortality. Both groups of animals, well-fed (indicated by + elsewhere in this paper) and semi-starved (indicated by -), were maintained in the same room, at the same temperature and humidity.

Larvae from both groups were examined in each instar for body length and width, ocelli number, number of segments, and gonopod development. Records were also kept of the duration of the instars, and the total number of instars to sexual maturity.

RESULTS

Body length and width

Length and width measurements (to the nearest 0.1 mm.) of specimens preserved two to three weeks following molting are presented in Figures 1 and 2. Length measurements were made from the tip of the head to the tip of the anal valve; width measurements were made by measuring the diameter of the fourth body segment.

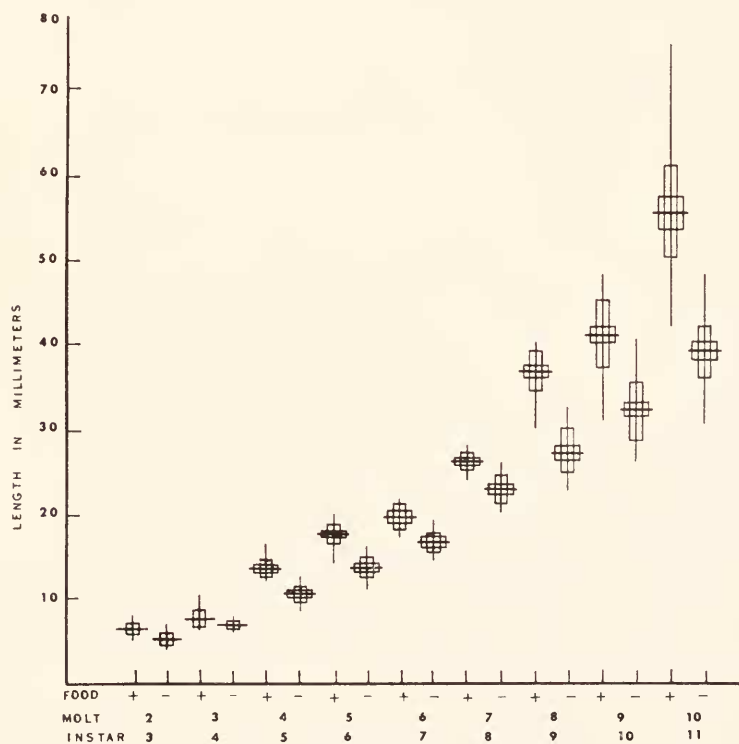


FIGURE 1. Length measurements of well-fed and semi-starved larvae.

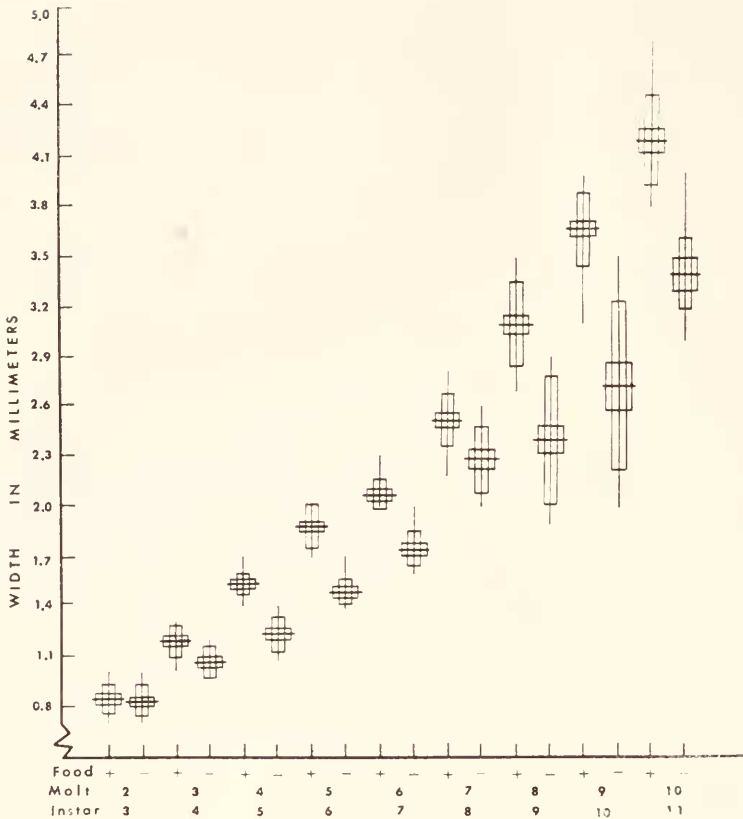


FIGURE 2. Width measurements of well-fed and semi-starved larvae.

The mean values for each instar, both length and width, are consistently lower for the semi-starved animals. In only two instars are the values not statistically different: the mean width of instars 3(+) and 3(-), and instars 4(+) and 4(-). All other means are significantly different at the p 0.001 level (t test), except for length in instars 3(+) and 3(-), and in 4(+) and 4(-), which are significant at the p 0.005 level.

It is clear that diet can affect the overall size of the animal, and therefore these two measurements (length and width) are not satisfactory indices for determining the correct instar. For example, the mean length and the distribution of length values for instar 11(-) are actually less than those for instar 10(+). The mean width of instar 9(-) is less than the mean width of instar 8(+). These are only two examples; other cases of overlap are evident in Figures 1 and 2.

Total number of body segments

The total number of body segments for both well-fed and semi-starved animals is presented in Figure 3 and Table I. The general pattern of segment increase is

similar in both groups of animals. However, there appears to be a difference in the early rate of segment increase from instars 4 through 6; after instar 6 the difference between the two groups does not increase. In fact, there is a slight convergence in instars 9 and 11.

Analysis of the mean number of segments per instar was carried out using the "t" test. Comparison of the means of the two groups demonstrates a statistical difference ($p < 0.001$) in instars 5 through 11. There are instances where the mean segment number for an instar of semi-starved larvae is actually less than the mean for an earlier instar of well-fed larvae; for example, instar 11(-) is lower than 10(+). There is also considerable overlap in the ranges (Table I) of instars 9(-) and 8(+), 8(-) and 7(+), 7(-) and 6(+), and 6(-) and 5(+).

Adults of both the semi-starved and well-fed groups are within the normal range of segment number for the species (51-59; Keeton, 1960) even though the mean of the semi-starved (53.69) is less than the mean of the well-fed (54.91). The mean of the well-fed group is very close to the overall mean for the species, 55.1 (Keeton, 1960).



FIGURE 3. Mean total segment number of well-fed and semi-starved larvae.

TABLE I
Segment number per instar (well-fed +, semi-starved -)

Instar	Molt	Mean segment number	Standard deviation	Standard error mean	Range limits	N
3+	2	21.33	0.65	0.19	21-23	12
3-	2	21.00	0.0	0.0	21	13
4+	3	26.67	0.57	0.11	25-27	24
4-	3	26.54	0.80	0.16	25-28	24
5+	4	32.52	0.64	0.13	31-34	25
5-	4	30.92	0.69	0.14	30-32	24
6+	5	38.75	0.78	0.15	37-40	28
6-	5	35.40	1.10	0.25	33-37	20
7+	6	44.10	0.82	0.14	43-46	30
7-	6	41.11	1.40	0.23	38-44	35
8+	7	48.30	0.93	0.19	46-50	23
8-	7	45.52	0.92	0.18	43-48	27
9+	8	51.65	1.40	0.32	49-54	20
9-	8	50.10	1.17	0.26	48-53	19
10+	9	53.77	1.35	0.26	51-57	26
10-	9	51.65	1.74	0.43	49-54	17
11+	10	54.91	1.34	0.28	52-57	22
11-	10	53.69	1.32	0.26	51-55	26

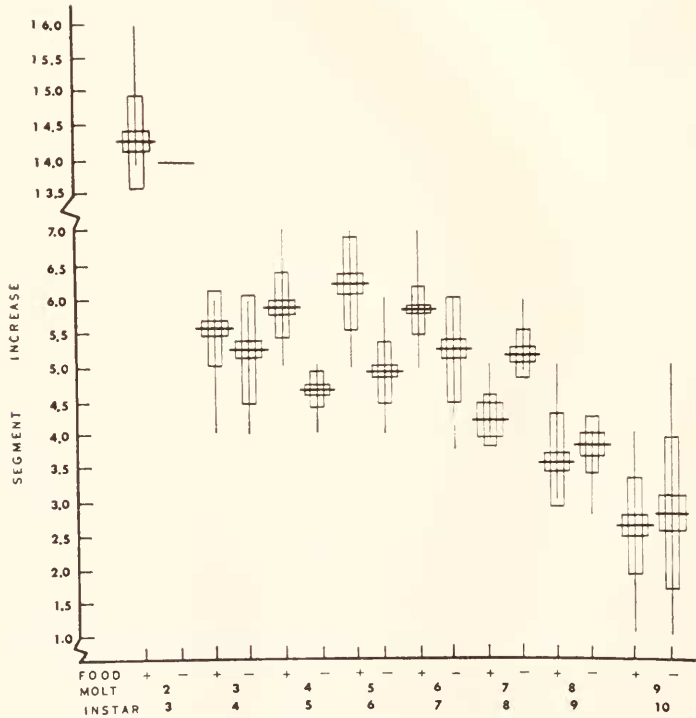


FIGURE 4. Segment increase per instar of well-fed and semi-starved larvae.

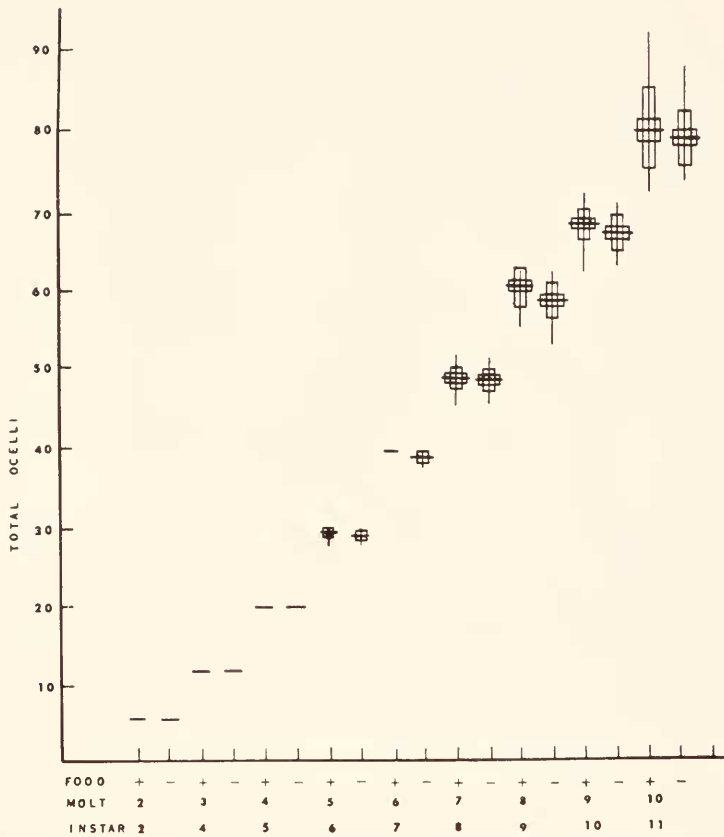


FIGURE 5. Ocelli increase per instar of well-fed and semi-starved larvae.

Segments added per instar

The effect of diet on segment addition is presented in Figure 4. At molts 4, 5, and 6, the well-fed larvae added more segments than the semi-starved (t test, $p < 0.001$). At molt 7, however, the semi-starved larvae added more segments than the well-fed ones ($p < 0.001$); the same trend was apparent at molts 8 and 9, though the difference was not statistically significant ($p < 0.3$ and 0.5).

The new segments develop from a posteriorly located proliferative region (located between the penultimate segment and the anal segment) during the first few days of the instar preceding the molt at which they first become outwardly visible (this was confirmed histologically, and will be the topic of another paper). For example, new segments exposed by the molt (molt 4) to instar 5 actually developed during the first three days of instar 4. Consequently, since the early instar larvae are enclosed within the egg capsule until the fourth day of instar 3, dietary effects on segment addition could not be detected until the molt to instar 5; the segments added at molt 3 (from instar 3 to 4) were originally determined during the first few days of instar 3. At this time both starved and well-fed larvae were still in the egg capsule.

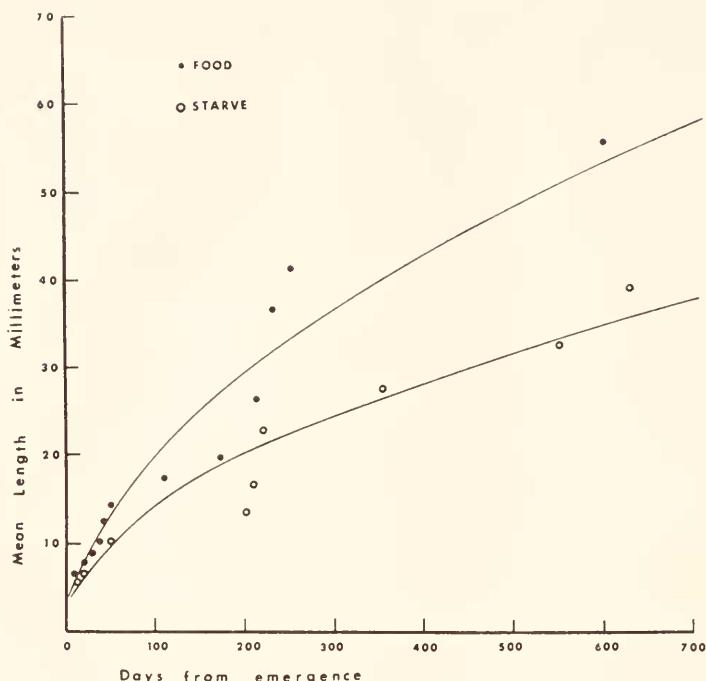


FIGURE 6. Mean body length in time of well-fed and semi-starved larvae. Each point is the mean of at least twenty animals.

Number of ocelli

Data on the number of ocelli characteristic of each instar are presented in Figure 5. In no instar is there a statistical difference between the means of the well-fed and the starved animals. In instars 3, 4, and 5, the means of the two groups are identical; the means of the remaining instars are practically the same. However, it is interesting to note that when there is a difference, the starved animals always have the lower means. Of particular significance is the lack of overlap in the ranges of the ocelli numbers for the different instars. Though the variability of ocelli number increases in the later instars, the ranges remain discrete.

A linear relationship between ocelli and number of molts is evident in Figure 5. It is not implied, however, that the correlation is between time and ocelli. On the graph, the instars are equally spaced; in reality, the time between instars is irregular and often not constant for animals in the same instar.

Number of instars

The several maturational sequences observed for *N. annularis* are shown in Table II. Though the most frequent sequence for both the well-fed and the semi-starved animals was type 3, in which there are 9 segment-building instars, two maturational instars, and maturity following the molt to instar 12, several variations were found. In particular, 25 semi-starved animals went through one

three or more instars. Box No. 20 had animals from instar 7 through instar 10, at 535 days from emergence. Six of 13 boxes of well-fed animals contained larvae in two instars; no boxes had animals in more than two instars. At 535 days, three animals in the well-fed group (box 12) were in instar 11, while one animal in the semi-starved group (box 20) was in instar 7. At this age, then, animals were in any of five different instars, almost half the total number of instars the animal goes through. Similarly, at about 180 days there was a four-instar spread, and equally large spreads were found for other ages.

In an attempt to get some index of development in time, data are presented plotting body length against age in days (Fig. 6). It appears that both groups (semi-starved and well-fed) have similar growth curves even though the starved are always lower than the fed. However, the periods between 100 and 250 days

TABLE IV
Box variability of well-fed millipeds

Box no.	Days from emergence	Instar								
		3	4	5	6	7	8	9	10	11
1	20	0	24							
2	40			24						
3	110				28					
4	173					25				
5	180					5	5			
6	209						7	1		
7	212						8			
8	246						4	9		
9	253							7		
10	265							2	14	
11	533							9		
12	535								10	3
13	549								4	14

are very irregular. In order to make a more meaningful comparison the data have also been plotted on double-log paper (Fig. 7).

The above data are only good enough to point out trends and trend differences between the two groups. The data are not good enough to be useful in predicting size changes.

Maturation of gonopods

The male copulatory organs, the gonopods, undergo definite morphological changes as the millipede passes through the instars. Regardless of the size of the animal, the complexity of the gonopods for a given instar is always the same; it is, therefore, possible to characterize an instar by the morphology of the male copulatory organs (Bernes, in press). It must be pointed out, however, that examination of the gonopods of a living larva often results in permanent injury or death. Furthermore, because of the simplicity of the female copulatory apparatus, gonopod staging is only possible in males.

DISCUSSION

From the preceding data it is clear that diet affects both the growth and morphogenetic processes that occur during the larval development of *N. annularis*. The growth effects are reflected in both length and width. However, since an effect on the number of segments is also demonstrated, one cannot fully attribute the length differences to growth alone; one might expect animals with fewer segments to be shorter. Width, however, is a good index of growth.

Diet has an effect on the number of segments built during an instar and on the number of segment-building instars. Semi-starvation results in addition of fewer than the normal number of segments in the early instars, and greater than

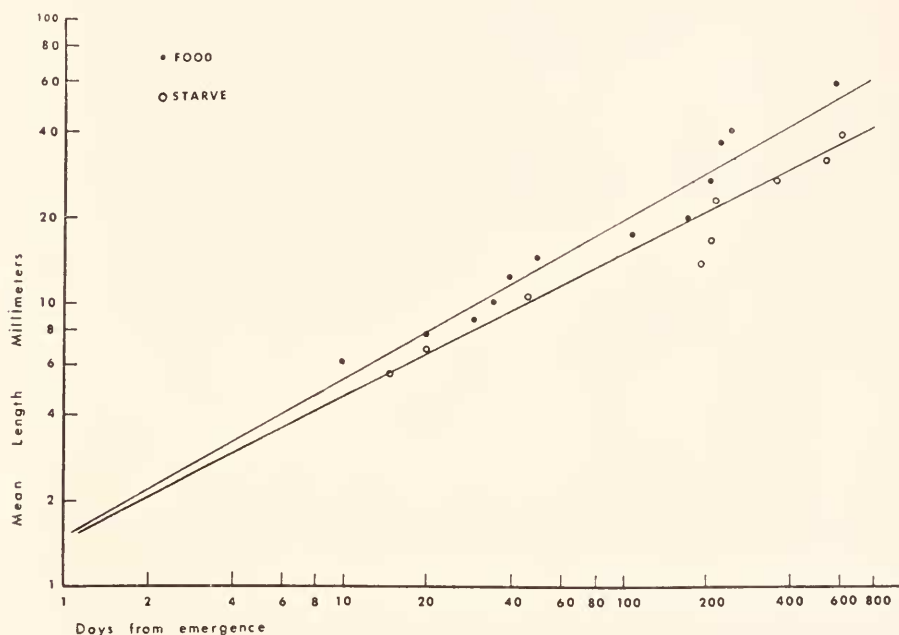


FIGURE 7. Double log plot of mean body length in time of well-fed and semi-starved larvae. These are the same data as plotted in Figure 6.

normal number in later instars. The increase of segment-building in the later instars, even though the animals are still on a semi-starvation diet, reflects the regulatory ability of the milliped segment-building system, which is also seen in the addition of extra segment-building instars by a significant number of semi-starved animals. The result of the segment regulation, whether by increased segment-building in the later instars, or by the addition of extra segment-building instars, was that semi-starved animals achieved a total number within the normal range for the species. However, because of the effects of diet, segment number cannot be used to characterize larval instars, despite the fact that this procedure has been used by most workers in the past (Loomis, 1933; Causey, 1955; Keeton, 1960).

The lack of a definite number of instars is not surprising in view of similar irregularity in other arthropods, but the addition of the extra instars in the milliped appears to be related primarily to prolonging the morphogenetic process of segment-building, rather than to attaining of a required size. In fact the data demonstrate that starved millipeds mature regardless of size, as long as the segment number is within the normal range. Nutrition has been shown to influence the growth rate and the number of ecdyses of several insects: *Blatella* (Seamans and Woodruff, 1939), *Tincola* (Titschack, 1926), and *Bombyx* (Bounhiol, 1938). In these cases, however, size seems to be the critical factor for maturation.

Effects of other environmental factors on the growth and molting of arthropods are well documented (Wigglesworth, 1965). Factors such as temperature have been shown to both increase the number of molts of *Sphodromantis* (Przibram and Megusar, 1912), *Dermestes* (Kreyenberg, 1929), and *Tenebrio* (Ludwig, 1956), or decrease the number of molts in *Melanophus* and *Picris* (Hoskins and Craig, 1935). Humidity, likewise, can effect the number of molts in *Thermobia* (Sweetman, 1934).

Not only is the number of instars variable in the milliped, but the time spent within the instars is even more variable. Semi-starvation merely exposes the limits of this variability; at 535 days of age, animals could be found in any of five different instars. Likewise, an attempt to correlate age with size was unsuccessful.

The only characteristics examined that were not significantly affected by diet were the number of ocelli and the morphological development of the male copulatory apparatus. In *Narceus annularis*, then, ocelli number is the most reliable staging characteristic in live specimens of both sexes.

SUMMARY

1. Growth, as measured by length and width, is substantially affected by diet. The length and width measurements were significantly lower for larvae in the semi-starved group.

2. The total number of body segments was statistically lower in the semi-starved larvae in instars 5 through 11. The difference was manifest at molts 5 and 6, remained constant at molts 7 and 9, and decreased slightly at molts 8 and 10.

3. Even though the mean number of segments was lower for adults of the semi-starved group, the range of adult segment number of the semi-starved animals was the same as the well-fed group and the same as the range given for the species.

4. Starvation caused the addition of fewer than normal (as compared to the well-fed group) segments in the early instars and a greater than normal number of segments in the later instars. Similarly, a significant number of semi-starved animals passed through either one or two extra segment-building instars.

5. The time spent in instars 4 through 11 was so variable that it was impossible to establish an index of development in time. Similarly, growth in time was most variable.

6. Diet did not have a significant effect on the number of ocelli added in each instar, or on the maturation of the male copulatory organs. These two characteristics are suitable characteristics for determining the instar of larvae, though the gonopods are difficult to examine in living specimens.

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